Factors Which Affect the Stability of Highly Unsaturated Fatty Acids.[1,2] I. Differences in the Oxidation of Conjugated and Nonconjugated Linoleic Acid

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STUDIES involving the initial stages of autoxidation of unsaturated fatty acids have indicated that oxygen might add directly across the double bond (1, 2) or at the "active" methylene groups (3, 4, 5) adjacent to the double bond. Farmer and co-workers (6, 7) have shown that oxygen adds across the double bonds in a conjugated system. However, the initial point of addition to a nonconjugated system is still open to question. Hilditch and co-workers (1, 2) believe that oxygen adds to the double bond, the bond shifts and hydroperoxides are formed. On the other hand, Bergstrom (3) and Farmer and co-workers (4) suggested that oxygen added directly onto the active methylene group and formed hydroperoxides at that point. Farmer (8) has recently modified this mechanism and suggested that oxygen may add to the double bond momentarily before forming hydroperoxides at the active methylene groups.

It would seem that by comparing the results obtained from the simultaneous oxidation and hydrogenation of pure nonconjugated and conjugated linoleic acid it might be possible to determine the site of hydroperoxide formation. In the present paper such comparisons were made with the use of $\Delta^{9,12}$ and $\Delta^{10,12}$ methyl linoleate and $\Delta^{9,12}$ and $\Delta^{10,12}$ linoleic or alkali conjugated linoleic acid.

Experimental

Preparation of Materials: All materials were prepared according to methods already described in the literature. The $\Delta^{9,12}$ linoleic acid or its methyl ester was prepared from corn oil [4] according to the method of Rollet (9), the alkali conjugated linoleic acid was prepared according to the method of Holman and Elmer (10) and the $\Delta^{10,12}$ linoleic acid or its methyl ester was prepared from dehydrated castor oil [5] according to the method of Von Mikusch (11).

The iodine value of the $\Delta^{9,12}$ linoleic and the specific absorption coefficient of the alkali conjugated and the $\Delta^{10,12}$ linoleic acid indicated that they had a purity of 98 and 97% of the theoretical value respectively.

Oxidation Procedure: Two oxidation procedures were used. In one, $\Delta^{9,12}$ or $\Delta^{10,12}$ methyl linoleate was oxidized in a closed system at 30°C. The apparatus was similar to the semi-micro hydrogenator shown in Figure 1 except that the flask and burette of the latter were smaller. A 200 ml. flask was connected by a flexible joint to a 100 ml. burette and a mercury-filled leveling bulb. The flexible joint made possible the shaking of the flask by a motor-driven eccentric while the rest of the apparatus remained stationary. Approximately 20 g. of the ester was weighed into the flask, the burette and flask swept with oxygen, and the oxygen brought to the lower level in the burette by opening the stopcock in the flask momentarily. The temperature and barometric pressure were then noted and the agitation of the flask started. The agitation was stopped at various intervals, the barometric pressure, temperature, and burette reading noted, and a sample removed for peroxide determinations, spectrophotometric readings, and hydrogenation. The flask was again swept with oxygen, the burette leveled as before, and the reaction allowed to continue. At each interval the initial and final volume of oxygen was also noted and corrected to standard conditions. By subtraction, the volume absorbed was determined and calculated as moles of oxygen per mole ester. Except for the removal of samples the reactions were carried out without interruption in a constant temperature room at 30° ± 1°C.

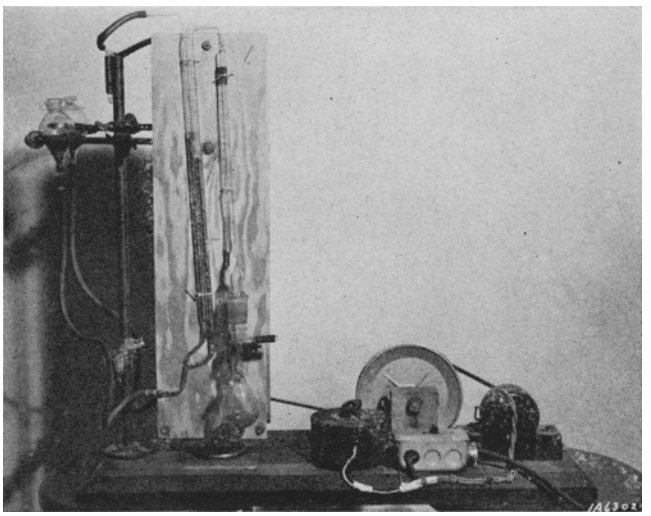

FIG. 1. Semi Micro Hydrogenation apparatus.

The other oxidation procedure was conducted in open 6″ test tubes at 30, 65, and 90°C. Tank oxygen was bubbled into the samples through glass inlet tips which led into the bottom of the test tubes. The latter were suspended in a 12″ by 12″ insulated Pyrex glass jar which contained light mineral oil (Figure 2), the temperature was regulated to a constancy of ±0.5°C. by an Aminco thermostatically-controlled heating unit. At appropriate intervals of time samples were removed for peroxide determinations, spectrophotometric readings, and hydrogenation.

[1] The subject matter of this paper has been undertaken in cooperation with the Office of Naval Research. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.

[2] Contribution No. 351 from the Department of Chemistry. A preliminary report of the work was given at the American Chemists' Society meeting in October, 1947.

[3] Portion of a thesis presented by Robert R. Allen as partial fulfillment of the requirements for the degree of Master of Science.

[4] A degummed corn oil furnished through the courtesy of Corn Products Refining Company, Argo, Ill.

[5] G. H. Dehydrated Castor Oil obtained through the courtesy of A. Eisenschmil, The Scientific Oil Compounding Company, Chicago, Ill.

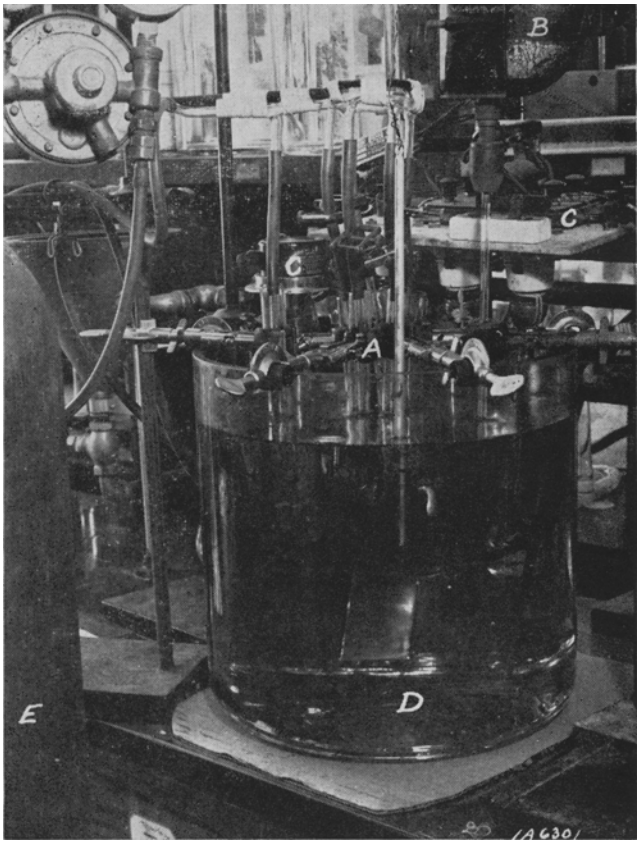

FIG. 2. Constant temperature bath. (A) 6" test tubes; (B) electric motor; (C) Amineo thermostat; (C) heater; (D) oil bath and; (E) tank oxygen.

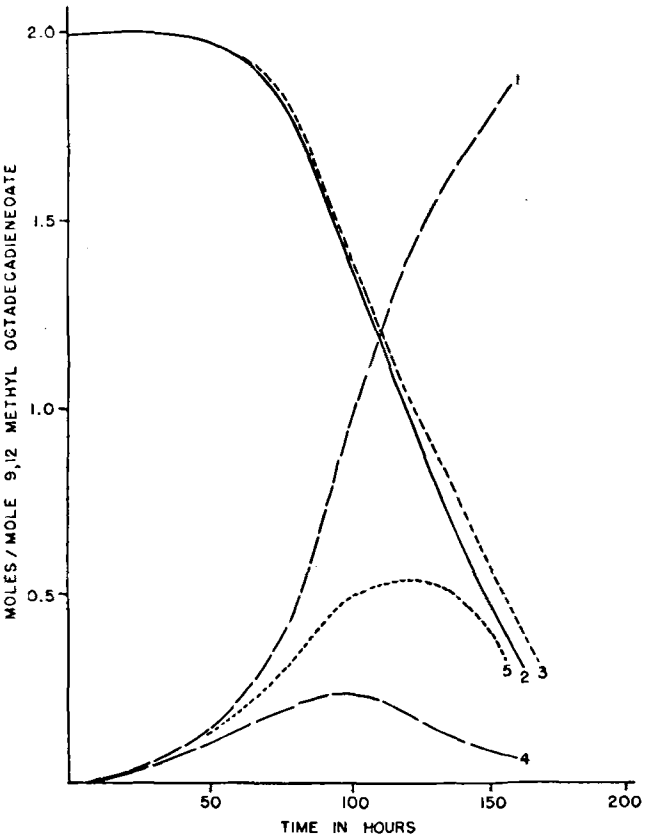

FIG. 3. The changes in characteristics in moles per mole of $\Delta^{9,12}$ methyl linoleate during autoxidation at 30°C. (1) total oxygen; (2) hydrogen corrected for peroxide oxygen; (3) 2 minus non-peroxide oxygen; (4) total conjugated; (5) peroxide oxygen.

Analytical Procedures: Hydrogen numbers (moles of hydrogen per mole of ester) were determined by means of a semi-micro modification (Figure 1) of the apparatus described by Johns and Seiferle (12). In this modification a larger reaction flask with a 25 instead of a 5 ml. burette was used to measure the volume of hydrogen absorbed. Samples of 40 to 100 mg. were hydrogenated with the aid of platinum on zirconium oxide as a catalyst.

The specific absorption coefficient of the oxidized ester was determined at 2320 Å on 100 mg. samples diluted with purified ethyl alcohol. The percentage conjugated ($a/115$) was calculated from the specific absorption coefficient according to the method of Kraybill et al. (13).

Peroxide oxygen was determined by the method of Wheeler (14). Duplicate samples of 100 mg. were removed, titrated immediately, and the results calculated as peroxide values or as moles of peroxide oxygen per mole ester.

Results and Discussion

The present results seem to substantiate the original mechanism of Farmer and co-workers (5). That is, the α methylene groups, rather than the double bonds, are initially attacked during the autoxidation of a nonconjugated ester. This was best shown by the sample which had been oxidized continuously in a closed system at 30°C. (Figure 3). Up to the point at which approximately 0.2 of a mole of oxygen had been absorbed, all of the oxygen could be demonstrated as peroxide oxygen and according to Bolland and Koch (15) all in the form of hydroperoxides. The latter could not have been added to the double bonds as over two moles of hydrogen per mole of methyl linoleate were still taken up even after as much as 0.2 of a mole of oxygen had been absorbed. However, after this point an increasing difference was noted between the total oxygen absorbed and the peroxide oxygen. The decrease in hydrogen values indicated that this difference, or non-peroxide oxygen, added to the double bonds to form a non-reductible compound. The peroxides, as such, were reduced quantitatively by hydrogen. The hydrogen values shown in Figure 3 and Figure 4 have been corrected for peroxide by subtracting the moles of peroxide from the moles of hydrogen actually absorbed.

The autoxidation of the conjugated ester in a closed system at 30°C. proceeded almost three times more slowly and in a different manner than the nonconjugated acid (Figure 4). For example, it took 279 as compared with 103 hours to add one mole of oxygen to the $\Delta^{10,12}$ and $\Delta^{9,12}$ methyl linoleate respectively. Furthermore, in contrast to the autoxidation of the nonconjugated ester the building up of a large amount of peroxide oxygen did not seem to be necessary as no peroxide oxygen was detected until $\Delta^{10,12}$ methyl linoleate had been oxidized for more than 100 hours. However, it was also possible that the peroxide oxygen was broken down as rapidly as it was being formed and that this oxygen then added to the double bonds. The fact that the decrease in hydrogen value

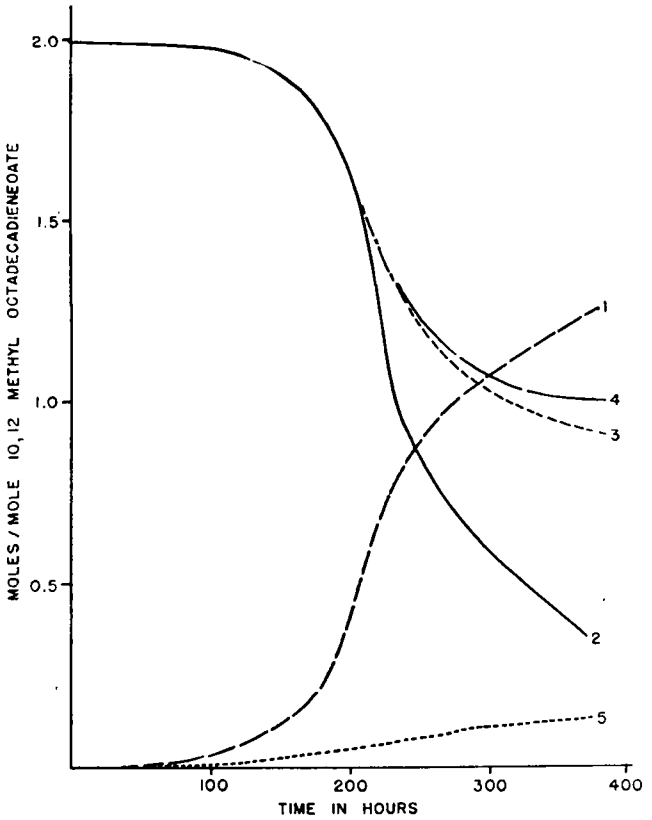

FIG. 4. The changes in characteristics in moles per mole of $\Delta^{10, 12}$ methyl linoleate during autoxidation at 30°C. (1) total oxygen; (2) hydrogen corrected for peroxide oxygen; (3) 2 minus non-peroxide oxygen; (4) 2 minus moles conjugated; (5) peroxide oxygen.

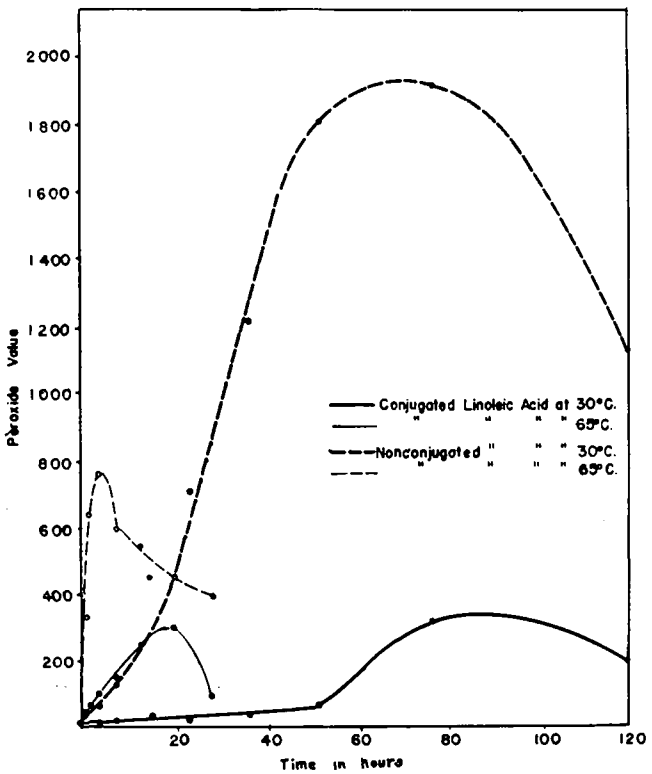

FIG. 5. The changes in peroxide values during the autoxidation of $\Delta^{9, 12}$ and $\Delta^{10, 12}$ linoleic acid at 30° and 65°C.

and the disappearance of conjugated double bonds were equivalent to the amount of oxygen taken up (Figure 4) indicated that oxygen-carbon rather than carbon to carbon polymerization (16) must have been occurring during the initial stages of the autoxidation at 30°C. The effect of higher temperatures on the total oxygen uptake and the rate and type of polymerization of $\Delta^{10, 12}$ methyl linoleate will be presented in a future publication.

In agreement with Gunstone and Hilditch (17) the present results indicated that the course of the autoxidation of $\Delta^{9, 12}$ methyl linoleate was independent of temperature. However, the rate and the amount of peroxide formed were dependent on the latter. The samples which had been oxidized in open test tubes at 30, 65, and 90°C. indicated that the maximum amount of peroxides (Figure 5) had been formed in 65, 5, and 3¼ hours respectively (Fig. 6). Further-

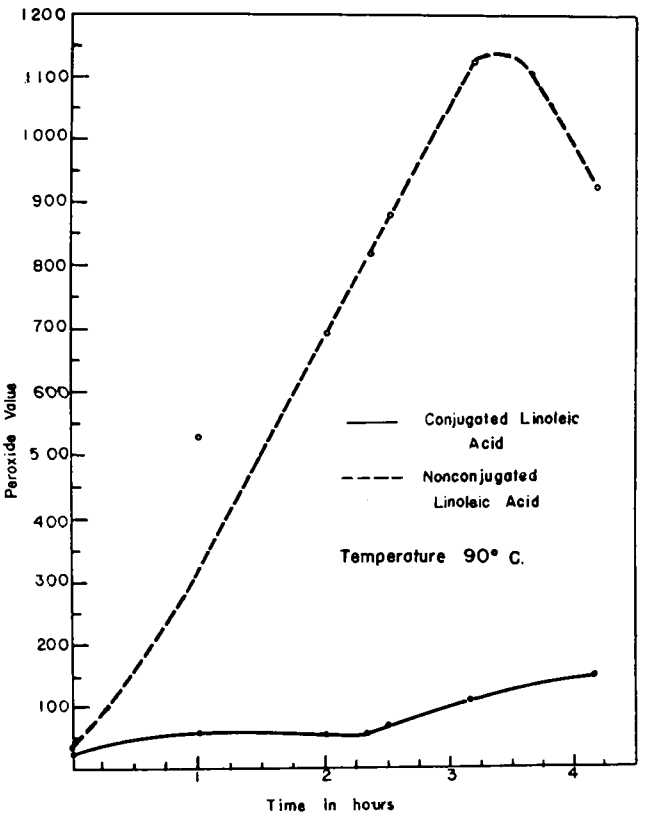

FIG. 6. The changes in peroxide values during the autoxidation of $\Delta^{9, 12}$ and $\Delta^{10, 12}$ linoleic acid at 90°C.

more at the maximum point of formation, a larger amount of peroxides was present at 30 than at 65 or 90°C. A similar trend in the formation of peroxides was noted during the autoxidation of the conjugated acid although from 8 to 10 times less peroxides were formed in the latter case.

The temperature at which the autoxidation of the nonconjugated acid was carried out also affected the rate and the extent of diene conjugation. The rate was more rapid at 65°C., but the specific absorption coefficient at 2320 Å indicated that the maximum amount of conjugation occurred at 30°C. (Figure 7). The specific absorption coefficient was 22 at 30°C., 18 at 65°C. (Figure 7), and 12 at 90°C. (Figure 8).

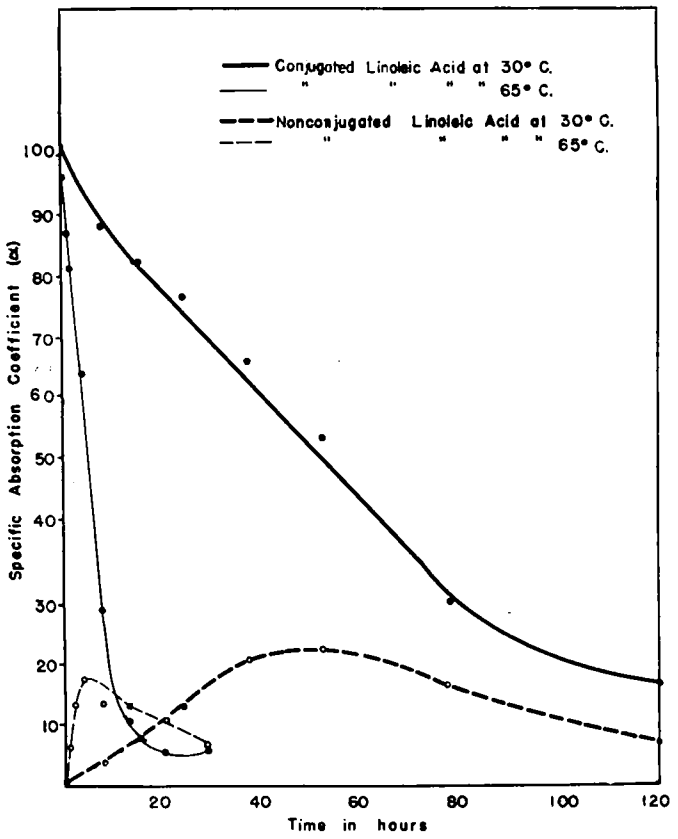

FIG. 7. The changes in the specific absorption coefficient of $\Delta^{9,12}$ and $\Delta^{10,12}$ linoleic acid oxidized at 30° and 65°C.

During the later stages of autoxidation the rate of decrease in conjugated bonds was approximately the same for both $\Delta^{9,12}$ or $\Delta^{10,12}$ linoleic acid. These changes were best reflected at 2320Å rather than at some other wave length (Figure 9). The magnitude

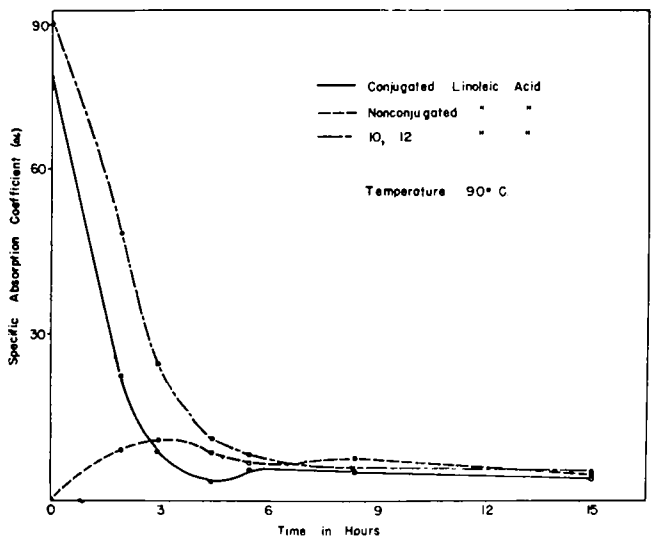

FIG. 8. The changes in the specific absorption coefficient at 90°C.

of the absorption at 2680 Å and 2775 Å indicated that very few secondary products were formed during the autoxidation of the methyl linoleate although such products have been reported by other workers (15, 18). The hydrogenation of samples removed from the acids which had been oxidized in open tubes yielded results similar to those obtained with the methyl esters which had been oxidized in the closed system at 30°C.

In a recent review (19) Hilditch pointed out that it required a large molar concentration of alkali hydroxide at a high temperature to rearrange the CH:CH CH_2 CH:CH linoleic system and that it was difficult to see why molecular oxygen at ordinary temperature should be capable of effecting a similar dissociation of a hydrogen atom or a proton. On the other hand, Bolland and Koch (15) pointed out that the oxidation product of methyl linoleate contained between 70-85% conjugated isomers and that up to 100% conjugation might occur. Holman (20) has also shown that the monohydroperoxide formed from linoleic acid during autoxiation is completely conjugated at least as far as 30% oxidation at 0°C. Therefore the extent of conjugation was greater than when alkali hydroxide is used although the rate of conjuga-

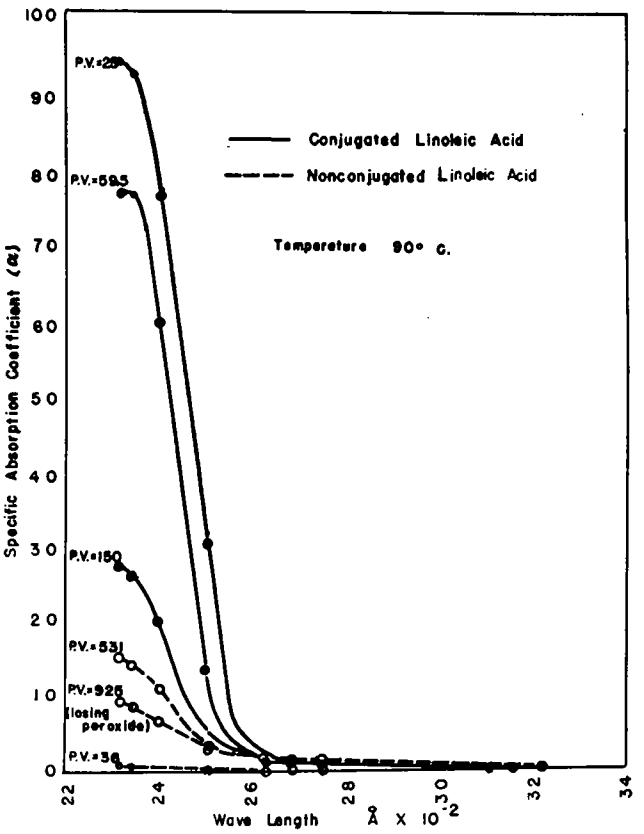

FIG. 9. The changes in the specific absorption coefficient at various wave lengths.

tion is faster in the latter case. Furthermore the present results indicate that the rearrangement of the nonconjugated acid is as extensive at 30 as at 90°C. The temperature at which the autoxidation is carried out is, therefore, not a primary factor. Although proof for the energy relationships is still lacking (8), the present results seem to indicate that oxygen in the form of hydroperoxide is as powerful a proton acceptor as alkali hydroxide.

Summary

The autoxidation of $\Delta^{9,12}$ and $\Delta^{10,12}$ methyl linoleate or the acids of these methyl esters was carried out

under various conditions and the changes in characteristics compared by the removal of small samples at selected intervals of time. The results indicated that during the initial stages of autoxidation of $\Delta^{9, 12}$ methyl linoleate at 30°C. all of the oxygen could be demonstrated as peroxide oxygen. On the other hand, no peroxide oxygen was former until the $\Delta^{10, 12}$ methyl linoleate had been oxidized for more than 100 hours. Furthermore, it was suggested that oxygen at room temperature was as effective in rearranging the CH:CH CH_2 CH:CH system as alkali hydroxide and high temperature.

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Report of the Spectroscopy Committee

November 15, 1948

AT the special meeting of the Spectroscopy Committee, held in Chicago during the 1947 Fall Meeting of the American Oil Chemists' Society, the spectrophotometric method for the analysis of fats and oils was discussed in detail. A few minor revisions were made. It was decided to analyze four oil samples (linseed, soybean, cottonseed, and lard) by the revised method before submitting it to the Uniform Methods Committee for action by the Society. In order to limit the amount of work necessary a simplified set of calculations was attached, in which the background corrections were eliminated. The calculations are as follows:

Absorption coefficient is defined as $k = D/bc$ where D is the observed spectral density of a solution of thickness b cm. (compared with solvent of the same thickness) and of concentration of c grams per liter, the concentration of c is equal to W/v, where W is the weight of sample in grams, and v is the total volume of solution in liters (0.1 the initial volume used times dilution factor). In the equations which follow subscripts 233, 268, etc., refer to wave length.

k = absorption coefficient before isomerization.
k' = absorption coefficient of isomerized materials.
$C_2 = (k_{233})\ 0.8403$ = % conjugated diene.
X = % arachidonic acid $= (k'_{316})\ 4.424$.
Y = % linolenic acid $= (k'_{268} - 0.534\ X)\ 1.880$.
Z = % linoleic acid $= (k'_{233} - k_{233} - 0.593\ X - 0.60\ Y)\ 1.124$.

Eight collaborators analyzed the four oils following the details of the method as closely as it was possible, in the individual laboratories. The variations were minor. The data obtained are shown in Tables I and II.

TABLE I

Collaborator	% Conj. Diene	% Acid Arach.	% Acid Lino-lenic	% Acid Linoleic
Soybean Oil				
1	.19	.23	8.17	53.2
2	.20	.18	8.20	53.0
3	.19	.16	7.87	53.8
4	.20	.19	8.36	55.4
5	.19	.22	8.42	53.7
6	.20	.27	9.15	55.8
7	.20	....	8.93	55.2
8	.20	.29	8.06	49.7
Average	.20	.22	8.40	53.7
Linseed Oil				
1	.26	.42	48.5	16.1
2	.27	.35	48.0	16.8
3	.25	1.21*	44.9	17.2
4	.26	.41	48.0	18.0
5	.25	.50	49.5	16.0
6	.26	.49	53.2	17.4
7	.26	....	50.4	17.6
8	.28	.31	44.8	15.0
Average	.26	.41	48.4	16.8
Cottonseed Oil				
1	.14	.13	.15	51.1
2	.15	....	.29	51.0
3	.14	.09	.13	49.5
4	.16	.05	.16	52.0
5	.15	.09	.15	51.5
6	.16	.13	.12	54.4
7	.15	....	.26	52.3
8	.15	.20	.14	45.9
Average	.15	.12	.18	51.0
Lard				
1	.23	.52	.77	12.1
2	.23	.51	.71	12.2
4	.23	.55	.69	12.2
4	.25	.40	.78	11.0
5	.23	.51	.73	12.2
6	.26	.50	.81	12.2
7	.24	.52	.82	13.2
8	.25	.41	.94	10.8
Average	.24	.49	.78	12.0

* Not in average.

An examination of the data in Table I shows that exceptionally good checks were obtained by all collaborators, except No. 8, which appears to be low in all cases for linoleic acid. This would appear to be a consistent error which is occurring in the laboratory of that collaborator rather than a fault in the method of analysis itself. The percentages of arachidonic acid found by the short calculations are without question in error, since it is very doubtful if arachidonic acid occurs in soybean, linseed, or cottonseed oil. In Table II is shown a comparison of the average results in Table I, with the same samples analyzed in one of the laboratories using the long calculations. It should also be noted that the long calculations show a higher percentage of linoleic acid and a somewhat lower percentage of linolenic acid. While it cannot be stated with certainty that the differences in linolenic and linoleic acid are significant, the lower values for arachidonic obtained by the long calculations are certainly more correct. Hence, in the method which becomes a part of this report, the long calculations